Stop-flow studies of solute uptake in rat lungs

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Effros, R. M., R. Schapira, K. Presberg, K. Ozker, and E. R. Jacobs. Stop-flow studies of solute uptake in rat lungs. J. Appl. Physiol. 85(3): 986–992, 1998.—Stop-flow studies were used to characterize solute uptake in isolated rat lungs. These lungs were perfused at 8 or 34 ml/min for 10–28 s with solutions containing [22]NaCl, [36]Cl–, or [86]RbCl as indicators. Uptake of [3H]mannitol, [14C]urea, and [3HOH] was observed only when the lungs were loaded at high flow for short intervals. Decreases in [3HOH] concentrations in the pulmonary outflow could be used to identify the fraction of the collected samples that were within exchange vessels of the lung during the stop interval and may help determine the distribution of solute and water exchange along the pulmonary vasculature.

In these studies, isolated lungs were perfused with an intravascular indicator (FITC-dextran with a molecular weight of 2 x 10^6) (3). Flow was then discontinued, and intravascular pressures were increased to 20 cmH2O for 10 min. At the end of this time, a small volume of an isotonic solution containing [3HOH] was instilled into the air spaces to effectively label the fluid that was in the exchange sites of the lungs during the stop-flow interval. The fluid remaining in the vasculature was then flushed from the lungs into serial sample tubes, and concentrations of FITC-dextran were used to determine how much fluid had been lost from the vasculature by filtration. Comparison of net filtration of unlabeled water from and diffusion of [3HOH] into the collection samples suggested that significant filtration occurs from venous vessels in the lungs but not from arterial vessels. This approach represents an important advantage of using stop-flow studies in the lungs, since retrograde flow is not possible in the nephron.

There are a number of potential disadvantages to using instilled [3HOH] to label the exchange vessels in the lungs: it is difficult to ensure uniform delivery to the pulmonary capillaries, [3HOH] remaining in the air spaces may continue to equilibrate with the fluid used to flush the vasculature after the perfusate that was in the exchange volume has been collected, and instillation of fluid into the air spaces could alter regional lung perfusion. We proposed that an alternative method for labeling the pulmonary exchange volume would be to perfuse the lungs with a solute that is taken up and retained within the tissues during the stop interval (3). If virtually all the indicator were removed from the perfusate in the exchange vessels of the lung, then decreases in indicator concentration could be used to determine how much of each collection sample was in the exchange vessels during the stop interval. This, in turn, could help document the site of filtration and the sites of uptake or production of metabolites in the pulmonary circulation. We previously obtained evidence for efficient uptake of [201Tl]+ in perfused lungs (2), suggesting that this K+ analog could be used for this purpose. In the present study, [201Tl] and [86Rb] were evaluated as potential indicators for labeling the pulmonary vascular exchange volume in stop-flow studies, and we have compared their uptake with that of other indicators that diffuse into the pulmonary tissues.

METHODS

Experimental approach. Rat lungs were initially perfused at a constant rate with an unlabeled solution. After several minutes the perfusate was changed to a solution that contained [22]NaCl, as a reference intravascular indicator, and two or more of the following low-molecular-weight indicators: [3H]mannitol, [14C]urea, [3HOH], [86Rb]Cl, or [201Tl]Cl (Table 1).

RELATIVELY LITTLE IS KNOWN about the distribution of solute and water exchange across the arterial, capillary, and venous segments of the pulmonary vasculature. Differences in the morphology of these regions suggest that important differences might exist regarding the manner in which transport occurs in these vessels (9). Stop-flow studies have been used to characterize the longitudinal distribution of solute absorption and secretion in the nephron (4), but this approach has not been widely applied to the pulmonary vasculature. Stop-flow studies were used by Piiper (7) to monitor gas exchange in the lungs. Chinard (1) also referred to its use in a review published in 1980 to study 22Na+ and 36Cl– exchange in the pulmonary circulation. However, interpretation of the latter data was complicated by uncertainties concerning the volume of the collected perfusate in the vasculature during the stop-flow interval.

In a recent study we used anterograde and retrograde stop-flow experiments to compare the sites of filtration and diffusion in the pulmonary vasculature.
Table 1: Experimental protocols

<table>
<thead>
<tr>
<th>Stop Interval, s</th>
<th>Stop Pressure, cmH2O</th>
<th>K+, mmol/l</th>
<th>n</th>
<th>Indicators</th>
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<td>125I-albumin, 201Tl, [3H]mannitol, [14C]urea</td>
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<tr>
<td>300</td>
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<td>4</td>
<td>7</td>
<td>125I-albumin, 201Tl, 3HOH</td>
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<td>Fast-flow studies (34 ml/min)</td>
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<td>n, Number of experiments.</td>
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Stop-flow experiments were divided into three stages: 1) during the prestop (loading) interval the lungs were perfused at various flow rates with a physiological solution containing a mixture of the indicators; 2) flow was arrested and left atrial pressures were maintained at 0 or 10 cmH2O during the stop interval, and 3) the fluid that was in the lungs during the stop interval was flushed out during the poststop (flush) interval by resuming perfusion with the labeled solution.

As indicated in Table 1, two sets of experiments were conducted. In the first set of studies the lungs were perfused at a relatively slow flow (8 ml/min) for 22.6–86 s, and flow was stopped for various intervals ranging from 10 to 300 s. Flow was then resumed at 8 ml/min to flush out the fluid that had been in the pulmonary exchange vessels during the stop-flow interval. In this initial set of experiments the lungs were perfused with fluid that was kept in a reservoir at 20 cmH2O above the lung. To keep the rate of flow constant during the loading and flush intervals, the outflow was pumped from the pulmonary artery. Samples were collected from the outflow at 0.42-s intervals. Several variables were studied with this protocol: K+ concentrations were varied between 1 and 40 meq/l, left atrial pressures were decreased from 10 to 0 cmH2O during the stop interval, and the duration of the stop flow was increased from 90 to 300 s.

In the second set of experiments a much more rapid rate of flow (34 ml/min) was used during the loading and flush periods, and each of these periods was only 10 s in duration. Perfusate was pumped with a peristaltic pump directly into the lungs at a constant rate, and pressures were measured in the pulmonary artery. Samples were collected from the outflow at 0.42-s intervals. Several variables were studied with this protocol: K+ concentrations were varied between 1 and 40 meq/l, left atrial pressures were decreased from 10 to 0 cmH2O during the stop interval, and the duration of the stop flow was increased from 90 to 300 s.

Surgical procedure. Lungs were harvested from 52 Sprague-Dawley rats [average weight 441 ± 61 and 476 ± 71 (SD) g in low- and high-flow studies, respectively, P = NS]. The rats were anesthetized to deep pain with an intraperitoneal injection of 0.6–0.75 ml of 65 mg/ml pentobarbital sodium. After anesthetization the chest was opened and the rat was anticoagulated with an intracardiac injection of 0.4 ml of 1,000 U/ml heparin sulfate. The trachea was intubated, and catheters were placed in the pulmonary artery and left atrium before excision of the heart and lungs from the chest. The lungs were kept inflated with 95% O2-5% CO2 at a pressure of 5 cmH2O.

Perfusate solutions and lung weights. The initial, unlabeled perfusate solutions contained 135 mM Na+, 120 mM Cl−, 25 mM HCO3−, 4 mM K+, 2.5 mM Ca2+, 0.8 mM Mg2+, 5 g/dl BSA, 150 mg/dl glucose, 10 mg/dl urea, and 5 mg/dl mannitol. The labeled solution was made by adding to each milliliter of the unlabeled perfusate three or more of the following radionuclides: 0.05 µCi 125I-albumin (used in all experiments, human; Mallinckrodt, St. Louis, MO), 0.2 µCi 201Tl (used in all experiments), 0.2 µCi [3H]mannitol, 0.2 µCi [14C]urea, 0.2 µCi 86Rb, and 0.8 µCi 3HOH (New England Nuclear, Wilmington, DE). More than 98% of the 125I-albumin radioactivity was retained by a 30,000-mol wt-cutoff Amicon filter, indicating that the 125I remained associated with the protein molecule. The pH of the fluids were adjusted to 7.4 with small volumes of 1 N NaOH in the presence of 94% O2-6% CO2, and P CO2, and pH were measured at 37°C. Gas concentrations were measured in two preparations (not included in Table 1) before and after the stop period. The pH remained constant, and P CO2 remained at 36–40 Torr. Po2 remained above 120 Torr. Increases in K+ concentrations were achieved by replacing NaCl with KCl in the perfusate solution.

After the experiments the lungs were weighed and dried at room temperature until weights were constant, and the wet-to-dry weight ratios were calculated. We found that drying these small lungs at room temperature for several days was equivalent to drying them for shorter intervals at 50°C.

Analysis and calculation. Aliquots (50 µl) of the outflow fluid were placed into 3 ml of scintillation fluid (LKB Optiphase Hisafe 3, Pharmacia) and counted first in a gamma counter (for 125I-albumin and 201Tl radioactivity) and then in a beta counter (for [3H]mannitol, [3H]mannitol, [14C]urea, and [3H]mannitol radioactivity). These counts were corrected for crossover and 201Tl decay, and the outflow counts per minute were divided by those in the arterial perfusion solution to yield outflow-to-inflow concentration ratios.

In the slow-flow studies, differences between the concentrations of the diffusible indicators and 125I-albumin were sufficiently great during loading and flushing that extravascular volumes (Vev) could be calculated for [3H]mannitol, [14C]urea, and 3HOH. The following equation was used for this purpose (Fig. 1, top trace) (2)

$$V_{evi} = \frac{b}{a} \sum_{j=a}^{b} \left( f_{albumin} - f_{i} \right) v_{j}$$ (1)

where i is the indicator, j is the sample number, a is the time at which the infusion solution is switched to the labeled solution, b is the time at which concentrations of the outflow and inflow have become the same, f is the outflow concentration divided by the inflow concentration (referred to as the “concentration ratio”), and vi is the volume of the jth sample, which is calculated from the product of the flow and the sampling interval. The area of the time-concentration curve that is used for calculating Vev of [3H]mannitol is
Fig. 1. Schematic diagram of volume (V) calculations. \( V_{\text{ev,mannitol}} \) was calculated as shaded area between \( ^{125}\text{I}\)-albumin and \([\text{H}]\)mannitol curves with Eq. 1. Similarly, \( V_{\text{ev,urea}} \) and \( V_{\text{ev,3HOH}} \) were calculated from areas between \( ^{125}\text{I}\)-albumin and urea and water curves (not shown). Fraction of each venous sample that was in exchange vessels of lungs during stop-flow interval \( (V_{\text{exch}}/V_{\text{ven}}) \) was calculated from observed and interpolated \( ^{201}\text{Tl} \) curves with Eq. 3. Total exchangeable volume in vasculature \( (V_{\text{exch}}) \) was calculated with Eq. 4. Values calculated for \( V_{\text{exch}} \) can equal true volume of exchange vessels only if indicator is completely removed from vasculature during stop-flow interval. In an antegrade flow study (flushed from artery to vein), 1st portions of completely removed from vasculature during stop-flow interval. In all experiments, concentration ratios for \( ^{201}\text{Tl} \) uptake. As indicated in Fig. 2, there was uptake of \( ^{201}\text{Tl} \) before and after the stop-flow interval in the slow-perfusion studies. Outflow concentration ratios of \( ^{201}\text{Tl} \) were below those of the vascular indicators \( ^{125}\text{I}\)-albumin and below those of \([\text{H}]\)mannitol and \([\text{14C}]\)urea, which each diffuses out of the vasculature into the pulmonary tissues. The decrease in \( ^{201}\text{Tl} \) concentrations after the stop flow was attributed to further uptake during the stop-flow interval. Increasing the duration of the stop-flow interval increased the calculated volume of perfusate that was cleared of \( ^{201}\text{Tl} \) (Fig. 3). However, prolonging the duration of the stop-flow interval from 90 to 300 s resulted in a relatively small increase in \( V_{\text{exch}} \) of \( ^{201}\text{Tl} \) in the slow- and the fast-flow experiments (see below). Fast perfusion: effects of K\(^+\) concentration, duration of stop flow, and left atrial pressures during stop interval. Outflow concentration ratios of \( ^{86}\text{Rb} \) were slightly greater than those of \( ^{201}\text{Tl} \) before and after the stop-flow period (Fig. 4). However, the relative decrease in concentrations after the stop-flow interval was similar when K\(^+\) concentrations in the perfusate were between 4 and 10 meq/l. This was reflected by similar clearances of \( ^{201}\text{Tl} \) and \( ^{86}\text{Rb} \) from the perfusate in each sample during the stop interval (indicated by equivalent values of \( V_{\text{exch}}/V_{\text{ven}} \) in Fig. 5) and calculated \( V_{\text{exch}} \) (2nd, 5th, and 6th sets of bars in Fig. 6; the apparent differences at 1 and 40 meq/l K\(^+\) were not
significant). Increasing the concentrations of K\(^+\) to 40 meq/l decreased \(V_{\text{exch}}\) of both indicators (Figs. 6 and 7). Concentration ratios of \(^{201}\text{Tl}\)\(^+\) were closer to those of each of other indicators, and concentrations fall significantly after stop-flow interval, in a manner consistent with additional uptake by tissues during stop-flow interval. Outflow concentrations of \(^{201}\text{Tl}\)\(^+\) remain well below those of \(^{125}\text{I}\)-albumin for a prolonged interval (inset), indicating that tissues had not become saturated with \(^{201}\text{Tl}\)\(^+\).

Mean values of \(V_{\text{exch}}\) of \(^{201}\text{Tl}\)\(^+\) in the fast-perfusion studies (0.629 ± 0.108 ml) were not significantly different from values in the control slow-flow studies (0.657 ± 0.034 ml). Increasing the stop interval from 90 to 300 s did not significantly increase \(V_{\text{exch}}\) of \(^{201}\text{Tl}\)\(^+\) or \(V_{\text{exch}}\) of \(^{86}\text{Rb}\)\(^+\) in these fast-perfusion studies.

The left atrial pressure was maintained at 10 cmH\(_2\)O in most of these experiments during the stop interval. In one series of experiments a comparison was made between the loss of indicators during the stop interval when left atrial pressures were set at 0 rather than at 10 cmH\(_2\)O during the stop interval (Fig. 5). Mean values of \(V_{\text{exch}}\) of \(^{86}\text{Rb}\)\(^+\) were significantly less at 0 than at 10 cmH\(_2\)O, but differences were not quite significant for \(V_{\text{exch}}\) of \(^{201}\text{Tl}\)\(^+\) (Figs. 5 and 6).

Uptake of other small indicator molecules before and during stop flow. Concentration ratios of \([^{3}\text{H}]\)mannitol and \([^{14}\text{C}]\)urea were less than those of \(^{125}\text{I}\)-albumin during loading and flushing in the slow-flow experiments, indicating loss of these indicators from the vasculature during perfusion (Fig. 2). Losses of \([^{14}\text{C}]\)urea exceeded those of \([^{3}\text{H}]\)mannitol: the calculated values of \(V_{\text{exch}}\) of \([^{3}\text{H}]\)mannitol averaged 0.06 ± 0.01 ml (\(n = 16\)), whereas \(V_{\text{exch}}\) of \([^{14}\text{C}]\)urea averaged 0.13 ± 0.01 ml in these slow-flow experiments (\(P < 0.01\), by paired \(t\)-test).

Concentration ratios of \([^{14}\text{C}]\)urea or \([^{3}\text{H}]\)mannitol decreased after the stop-flow intervals when the lungs were perfused in the slow-flow experiments (which were perfused at 8 ml/min for >20 s, Fig. 2). However, when the rates of perfusion were increased to 34 ml/min and the lungs were perfused with the indicators for only 10 s, decreases were detectable in the concentra-
tions of [3H]mannitol, [14C]urea, and 3HOH (Figs. 4 and 8). Calculated values of $V_{exch}$ of [3H]mannitol averaged 43 ± 7% those of 201Tl$^+$ and $V_{exch}$ of [14C]urea averaged 33 ± 5% those of 201Tl$^+$. The relative decreases in 3HOH were small, and it was difficult to calculate a value for $V_{exch}$ of 3HOH.

Fig. 5. Decreasing left atrial pressure (LAP) during stop interval reduced apparent volume of exchangeable vessels ($V_{exch}$) in lung, reflected by lower values of $V_{exch}/V_{ven}$ in collected samples. Nearly all fluid in exchange volume of lung during stop interval was recovered during first 5 s after stop interval. $V_{ven}$, Volume of venous sample.

Pulmonary arterial pressures and lung water content. Pulmonary arterial pressures were kept at 20 cmH$_2$O in the slow-perfusion studies. Much higher pulmonary arterial pressures were observed in the fast-perfusion experiments: 35.0 ± 9.5 (SD) cmH$_2$O during the 10-s loading period in 19 experiments in which it was successfully measured and 32.1 ± 11 cmH$_2$O during the 10-s flush period in 18 experiments. Pulmonary arterial pressures were >50 cmH$_2$O during the loading and flush periods in two lungs perfused with 40 meq/l K$^+$ and two lungs perfused with 1 meq/l K$^+$. Lung weights were slightly but significantly greater at the end of the fast-perfusion experiments than in the slow-perfusion experiments: 1.90 ± 0.23 vs. 1.67 ± 0.18 (SE) g ($P < 0.01$). Similarly, wet-to-dry weight ratios were slightly

Fig. 6. Values of $V_{exch}$ in fast-perfusion experiments. Increasing perfusate K$^+$ concentrations to 40 meq/l significantly decreased $V_{exch}$ of 201Tl$^+$ and 86Rb$^+$. Increasing duration of stop interval to 300 s did not have significant effects on $V_{exch}$ for these indicators. $V_{exch}$ of 86Rb$^+$ was reduced when left atrial pressures were zero rather than 10 cmH$_2$O during stop intervals. $V_{exch}$, Indicator $V_{exch}$.

Fig. 7. Increasing concentration of K$^+$ in perfusate resulted in a decrease in fall of 201Tl$^+$ in samples collected after a 90-s stop-flow period. Decrease in uptake is attributed in part to a decrease in Nernst potential of pulmonary cells but may also reflect a decrease in exchangeable vascular volume (see DISCUSSION). $f_i$, Concentration ratio for indicator $i$. 

Fig. 8. Values of $V_{exch}$ in fast-perfusion experiments. Increasing perfusate K$^+$ concentrations to 40 meq/l significantly decreased $V_{exch}$ of 201Tl$^+$ and 86Rb$^+$. Increasing duration of stop interval to 300 s did not have significant effects on $V_{exch}$ for these indicators. $V_{exch}$ of 86Rb$^+$ was reduced when left atrial pressures were zero rather than 10 cmH$_2$O during stop intervals. $V_{exch}$, Indicator $V_{exch}$.
was resumed, extraction of $^{201}$Tl exchange portions of the vascular bed. As soon as flow had become saturated with this indicator but was uptake (Figs. 3 and 6). This was not because the tissues interval from 90 to 300 s had a relatively minor effect on is extended from 10 to 300 s, increasing the stop from the lungs decrease more when the stop-flow period did not have a significant effect on the loss of $^{201}$Tl K

As indicated in our earlier study (3), the first samples of the perfusate that were in exchange vessels during stop flow are derived from more venous portions of the vasculature than were the later samples when the lungs are perfused in an anterograde direction from artery to vein. During retrograde flushes the initial samples of the fluid would be derived from more arterial portions of the vasculature than were later samples. Retrograde experiments were not conducted in the present study but were reported previously, with $^3$HOH instilled into the air spaces as a marker for exchange vessels rather than $^{201}$Tl$^+$ or $^{86}$Rb$^+$ (3). Although the anatomic site of indicator exchange is not defined in experiments of this sort, it is possible to determine the relative locations of such exchange processes as diffusional exchange and edema formation and reabsorption utilizing stop-flow experiments (3), and it may also be possible to follow release and uptake or metabolism of mediators along the vasculature with this approach, much as solute uptake has been characterized in the nephron.

An ideal indicator of the exchange volume of the lung would be removed from the exchange vessels minimally during loading and flushing and maximally during the stop interval. Three types of indicators were tried in this experiment: K$^+$ analogs ($^{201}$Tl$^+$ and $^{86}$Rb$^+$), small solutes, which diffuse primarily into the pulmonary extravascular compartment, and again, it was difficult to detect any additional losses from the circulation actually occurred during the loading period, and at low perfusion rates it was difficult to detect any additional decrease in the concentrations of these indicators during the stop interval. $^3$HOH rapidly entered the extravascular compartment, and again, it was difficult to detect any additional losses from the circulation during the slow stop-flow experiments. In contrast, much more of the uptake of $^{201}$Tl$^+$ and $^{86}$Rb$^+$ occurred during the stop interval, because the cells represent a large reservoir for these indicators. Volatile agents represent a fourth type of indicator that could be tried to mark the exchange vessels of the lungs during.

3) Clearance of $^{201}$Tl$^+$ and $^{86}$Rb$^+$ from the perfusate during stop-flow studies was similar at physiological K$^+$ concentrations. As in the cardiac circulation (5), uptake of $^{201}$Tl$^+$ was slightly greater than that of $^{86}$Rb$^+$ during the loading and flush intervals, but values calculated with Eq. 4 for $V_{exch}$ with each of these indicators were not significantly different. Some of the decrease in uptake of $^{201}$Tl$^+$ and $^{86}$Rb$^+$ at very high concentrations of K$^+$ could also be related to vasoconstriction and dercruitment, which would be expected with hyperkalemia (10). Pulmonary arterial pressures did tend to be high at high and low K$^+$ concentrations.

If it can be assumed that concentrations of $^{201}$Tl$^+$, $^{86}$Rb$^+$, or comparable solutes fall to negligible values during stop-flow periods of sufficient duration, then the fraction ($V_{exch}/V_{ven}$) of each of the venous collection samples can be calculated with Eq. 3 and the total exchangeable volume in the pulmonary vasculature can be calculated with Eq. 4. As indicated in our earlier study (3), the first samples of the perfusate that were in exchange vessels during stop flow are derived from more venous portions of the vasculature than were the later samples when the lungs are perfused in an anterograde direction from artery to vein. During retrograde flushes the initial samples of the fluid would be derived from more arterial portions of the vasculature than were later samples. Retrograde experiments were not conducted in the present study but were reported previously, with $^3$HOH instilled into the air spaces as a marker for exchange vessels rather than $^{201}$Tl$^+$ or $^{86}$Rb$^+$ (3).

Fig. 8. Concentration ratios of $^3$HOH were significantly below those of $^{125}$I-albumin before stop flow in each of 4 experiments conducted at fast perfusion rates, indicating diffusion out of vasculature before stop interval. After a 90-s stop interval, a small decrease in $^3$HOH concentration was observed, indicating that additional $^3$HOH had equilibrated with pulmonary tissue during stop interval.

DISCUSSION

$^{201}$Tl$^+$ and $^{86}$Rb$^+$ are efficiently removed from the circulation of a wide variety of organs (2, 5, 6, 8). Because uptake of these indicators is similar to that of K$^+$, they become concentrated within the cellular compartment. In a recent study we showed that $^{201}$Tl$^+$ is progressively removed from the pulmonary circulation and that extravascular concentrations reach levels that are $\sim$18 times intravascular concentrations (2). Three observations in the present group of experiments are consistent with the conclusion that nearly all the $^{201}$Tl$^+$ is removed from the perfusate remaining in the exchange vessels during stop-flow experiments: 1) Although concentrations of $^{201}$Tl$^+$ in perfusate flushed from the lungs decrease more when the stop-flow period is extended from 10 to 300 s, increasing the stop interval from 90 to 300 s had a relatively minor effect on uptake (Figs. 3 and 6). This was not because the tissues had become saturated with this indicator but was presumably because there was very little left in the exchange portions of the vascular bed. As soon as flow was resumed, extraction of $^{201}$Tl$^+$ from the circulation returned to levels close to those that were observed before flow was discontinued (Figs. 3 and 6). This was not because the tissues had become saturated with this indicator but was presumably because there was very little left in the exchange portions of the vascular bed. As soon as flow was resumed, extraction of $^{201}$Tl$^+$ from the circulation returned to levels close to those that were observed before flow was discontinued. 2) Increasing K$^+$ concentrations in the perfusate should reduce the Nernst potential between the cellular and intracellular compartments, and uptake of $^{201}$Tl$^+$ and $^{86}$Rb$^+$ did decline when K$^+$ concentrations were increased to 40 meq/l. However, at $\leq$10 meq/l, changes in K$^+$ concentrations did not have a significant effect on the loss of $^{201}$Tl$^+$ from the venous samples, suggesting that over this range of K$^+$ levels there was very little $^{201}$Tl$^+$ left in the plasma.
stop-flow studies. For example, preliminary studies in our laboratory indicate that a large fraction of labeled acetone is lost from the vasculature during stop-flow intervals, presumably into the air spaces. Alternatively, metabolites that would be efficiently removed or degraded during a stop interval could be utilized.

The duration and speed of loading and flushing the perfusate from the lungs have an important bearing on the outcome of stop-flow experiments. In the slow-perfusion studies, loading and subsequent flushing occurred over a longer interval, and there was considerable uptake of indicators from the circulation before and after the stop-flow interval. This was particularly true for \(^{201}\text{Tl}^+\): concentration ratios of this indicator before and after the stop interval were considerably lower when the lungs were perfused at 8 than at 34 ml/min (cf. Figs. 2 and 4). However, despite greater extraction of \(^{201}\text{Tl}^+\) and \(^{86}\text{Rb}^+\) before and after the stop interval in the slow-perfusion studies, calculated values of \(V_{\text{exch}}\) of these indicators were not significantly influenced by the rates of loading and flushing.

\(V_{\text{exch}}\) of \(^{86}\text{Rb}^+\) appeared to be decreased when left atrial pressures were decreased to 0 from 10 cmH\(_2\)O (Figs. 5 and 6). This would be expected if the amount of perfusate in the exchange volume of the lung was reduced at lower left atrial pressures.

Decreases in concentrations of \(^{3}\text{H}\)mannitol, \(^{14}\text{C}\)urea, and \(^{3}\text{HOH}\) after the stop interval could only be observed when loading was rapid and the duration of loading was short. This suggests that rapid loading and flushing are advantageous if indicators of this nature are being investigated. However, excessive perfusion rates should be avoided during these intervals, since increases in pulmonary arterial pressures could be associated with pulmonary vascular injury. We found evidence for a small amount of edema in lungs briefly perfused at rapid rates.

It is likely that much of the \(^{201}\text{Tl}^+\) and \(^{86}\text{Rb}^+\) lost from the vasculature crosses the luminal surface of the pulmonary capillaries and enters the endothelial cells. Additional quantities of these indicators may diffuse through the intercellular junctions and enter endothelial, interstitial, and epithelial cells of the lungs. It is generally assumed that much of the \(^{14}\text{C}\)urea and \(^{3}\text{H}\)mannitol that is lost from the vasculature diffuses through the interendothelial junctions into the pulmonary interstitium, and comparable quantities of \(^{201}\text{Tl}^+\) and \(^{86}\text{Rb}^+\) may diffuse through these structures into the interstitium. Pulmonary edema caused by increased intravascular pressures or injury to the capillary wall may alter the manner in which \(^{201}\text{Tl}^+\) and \(^{86}\text{Rb}^+\) are removed from the pulmonary circulation and the quantity of fluid in the exchange vessels of the lungs. However, these circumstances would not invalidate the calculation of \(V_{\text{exch}}\) if cellular uptake continues to remove all the \(^{201}\text{Tl}^+\) and \(^{86}\text{Rb}^+\) remaining in the exchange sites of the lungs during the stop interval.

\(^{3}\text{HOH}\) rapidly crosses cellular membranes and equilibrates with water in the cellular and interstitial compartments. It has been generally assumed that the distribution of \(^{3}\text{HOH}\) between the vasculature and tissues of the lungs is limited by flow rather than by the permeability of the pulmonary vasculature to water. The small decreases in concentrations that were observed after the stop interval indicated that additional \(^{3}\text{HOH}\) had diffused out of the vessels after the 10-s loading period. This could represent some diffusion from perfused regions of the lungs to those that remained unperfused rather than a barrier at the capillary wall. In other words, the rate of diffusion of \(^{3}\text{HOH}\) across the capillary wall could in theory be virtually the same as that observed between tissue compartments that are not separated by barriers, but equilibration between compartments may be delayed by the time required for free diffusion to distant sites. Nevertheless, this observation does suggest that if tissue perfusion is not uniform, delivery of \(^{3}\text{HOH}\) to the lung tissues may be limited in part by diffusion rather than flow.

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